

Claims

1. A method of making an antibody molecule, the antibody containing an immunoglobulin heavy chain comprising a $\alpha 3$ domain or a mu domain, the method comprising:

- (a) Providing a nucleotide sequence encoding the immunoglobulin heavy chain;
- (b) Modifying the nucleotide sequence in the region of the nucleotide sequence encoding the C-terminus 18 amino acids of the completed heavy chain to remove, or reduce the effectiveness of, one or more vacuolar targeting signal sequences to form a modified nucleotide sequence;
- (c) Inserting the modified nucleotide sequence into a host cell; and
- (d) Causing the host cell to express the modified nucleotide sequence to form the modified antibody heavy chain and secrete the modified antibody heavy chain from the host cell.

2. A method according to claim 1 wherein the heavy chain molecule is IgA, IgM or an IgA/G hybrid.

3. A method according to claim 1 or claim 2, wherein nucleotide sequence is modified by one or more point mutations of the nucleotide sequence, deleting one or more nucleotides, adding one or more nucleotides and/or replacing one or more nucleotides with a synthetic nucleotide sequence.

4. A method according to claim 3, wherein the synthetic nucleotide sequence encodes an amino acid sequence of general formula:



where: C = a cysteine residue

Xaa₁ = independently any amino acid with the proviso that it is not from I, L or forms a consecutive sequence X₁ X₂ X₃ V S X₄

where: X₁ = N, H or L

X₂ = V or Y

X₃ = S or N

X₄ = aliphatic amino acid

Xaa₂ = independently any amino acid
m = at least 2
n = 0 to 5.

5. A method according to claim 4, wherein Xaa₂ is Y and n = 1.
6. A method according to any preceding claim, wherein nucleotides encoding the last 16 amino acids of the heavy chain are deleted.
7. A method according to any preceding claim wherein one nucleotides encoding an amino acid sequence having a formula:
- (a) SCMVGHEALPMNFTQKTIDRLSGKPACY,
 - (b) SCMVGHEALPMNFTQKTIDRLSGKPAAACY,
 - (c) SCMVGHEALPMNFTQKTIDRLSGKPHASTPEPDPVACY or
 - (d) SCMVGHEALPMNFTQKTIDRLSGKPAAAAACY
8. A method according to any preceding claim wherein the nucleotide sequence modified originally encoded the amino acid sequence:
- X₁ X₂ X₃ V S X₄
- where: X₁ = N, H or L
X₂ = V or Y
X₃ = S or N
X₄ = aliphatic amino acid.
9. A method according to claim 8, wherein the amino acid sequence is: N V S V S V.
10. A method according to any one of the preceding claims wherein the nucleotide sequence modified encoded L or I.
11. A method according to claim 10, wherein the modified amino acid is one or both of an isoleucine 3 amino acids and/or 10 amino acids from the C-terminus end of the completed heavy chain.
12. A method according to any preceding claim, wherein the nucleotide sequence modified is within the sequence:

P T X₁ X₂ X₃ V S X₄ X₅ X₆ X₇ X₈ X₉ X₁₀ X₁₁ X₁₂ C X₁₃

where: X₁ = N, H or L, preferably N

X₂ = V or Y, preferably V

X₃ = S or N

X₄ = an aliphatic amino acid, preferably V or L

X₅ = an aliphatic amino acid, preferably I, V or L

X₆ = M, V or L, especially M

X₇ = S or A

X₈ = E or D

X₉ = any amino acid, preferably G, V, A or T

X₁₀ = D, E, G or A, preferably D

X₁₁ = G or S, preferably G

X₁₂ = I, T, V, Z or A, preferably I or T

X₁₃ = may or may not be present and, where present is A or Y

13. A method of adding J-chain binding capability to the heavy chain of an antibody comprising the steps of:

(a) providing a nucleotide encoding an immunoglobulin heavy chain;

(b) adding to that nucleotide a nucleotide sequence encoding a synthetic tail with the amino acid sequence:

-(Xaa₁)_m C(Xaa₂)_n

where: C = Cys

Xaa₁ is independently any amino acid with the proviso that it is not I or L or forms a consecutive sequence X₁ X₂ X₃ V S X₄ (where X₁ = N, H or L; X₂ = V or Y; X₃ = S or N; X₄ = aliphatic amino acid)

Xaa₂ = independently any amino acid

m = at least 2

n = 0 to 5; and

(c) expressing the completed nucleotide in a host cell to form an immunoglobulin heavy chain capable of binding J-chain.

14. A method according to any preceding claim, wherein the host cell is a plant cell.

15. A method according to claim 14, wherein the plant cell is part of a transgenic plant.

16. A method according to any preceding claim additionally comprising the step of isolating and purifying the antibody molecule.

17. A method according to claim 16, wherein the antibody is subjected to a protease digest to for Fab or F(ab')₂ fragments.

18. An antibody obtainable by a method according to any preceding claim.

19. An antibody containing a heavy chain comprising an $\alpha 3$ domain or a mu domain, the $\alpha 3$ domain or mu domain lacking one or more targeting signals towards the C-terminal end.

20. An antibody capable of binding J-chain comprising at its C-terminal end the sequence:

$-(Xaa_1)_m C(Xaa_2)_n$

where: C = Cys

Xaa₁ is independently any amino acid with the proviso that it is not I or L or forms a consecutive sequence X₁ X₂ X₃ V S X₄ (where X₁ = N, H or L; X₂ = V or Y; X₃ = S or N; X₄ = aliphatic amino acid)

Xaa₂ = independently any amino acid

m = at least 2

n = 0 to 5

21. An antibody according to claims 18 to 20 which does not contain the targeting signal: X₁ X₂ X₃ V S X₄

where: X₁ = N, H or L

X₂ = V or Y

X₃ = S or N

X₄ = aliphatic amino acid.

22. An antibody according to claim 21, wherein the targeting signal is N V S V S V.

23. An antibody according to any one of claims 18 to 22 which contains one or no isoleucine or leucine amino acids within the last 18 amino acids at the C-terminus of the heavy chain of the antibody.

24. An antibody according to any one of claims 18 to 23 comprising at the C-terminus end of the heavy chain of antibody, the sequence:



where: C = cysteine residue

Xaa₁ = independently any amino acid with the proviso that it is not I or L
or forms a consecutive sequence X₁ X₂ X₃ V S X₄

where: X₁ = N, H or L

X₂ = V or Y

X₃ = S or N

X₄ = aliphatic amino acid

Xaa₂ = independently any amino acid

m = at least 2

n = 0 to 5.

25. An antibody according to any preceding claim in which at least two, preferably two to four, glycine or alanine residues are present downstream of a C-terminal targeting sequence

26. An antibody according to any preceding claim in which at least the terminal amino acid residue of a C-terminal targeting sequence is replaced by at least two, preferably two to four, glycine or alanine residues.

27. Use of an isolated antibody according to any one of claims 18 to 26 in the manufacture of a medicament to treat disease.

28. A method of treating a disease by administering an antibody according to any one of claims 18 to 26 to a patient.

29. A method of prophylaxis, comprising administering an antibody according to any one of claims 18 to 26 to a person or animal.

30. A vector comprising a nucleotide sequence encoding an antibody according to any one of claims 18 to 26.

31. A host cell comprising a nucleotide sequence encoding antibody according to any one of claims 18 to 26 or a vector according to claim 30.

32. A transgenic plant comprising a nucleotide encoding an antibody according to any one of claims 18 to 26.

33. An immunoassay comprising an antibody as defined in any one of claims 18 to 26